Phytoremediation of Crude Oil Polluted Soils Using Native plant Species, Organo-mineral fertilizer and Brassinolide in Acidic Ultisol, South-eastern Nigeria

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ABSTRACT

Greenhouse and field studies were conducted to evaluate the potentials of some native plant species for phytoremediation of crude oil polluted soils. Twelve plant species (Axonopus compressus, Pennisetum purpureum (PP), Eleusine indica, Panicum maximum, Leuceana leucocephala (LL), Gliricidia sepium, Talinum fructicosum, Chromoleana odorota, Cyperus rotundus, Calapogonium mucunoides, Jatropha curcas, Centrosema pubescens) were studied under four levels of crude oil pollution (0,2.5, 5.0 and 7.5 % (w/w) using a Completely Randomized Design. Two species (PP and LL) with considerable phytoremediation potentials from the green house experiment were further studied under field condition with four levels of crude oil, organo-mineral fertilizer (OF) (5 t/ha) and brassinolide (250 ml per plant). Treatments were laid into a Randomized Complete Block Design with three replications. Soil samples were collected and analysed for total hydrocarbon content. Results obtained from the screen house and field show that, there was a significant ($p<0.05$) reduction in total hydrocarbon content of the soil and an increase in its absorption by the plants. PP had the highest absorption efficiency of 79.8 %, followed by LL (61.0 %) against (41.0 %) in non-amended soils and (38.6 %) in non-vegetated soil. The possible reasons for the high uptake of petroleum hydrocarbon by these two plants were due to such mechanisms as rhizodegradation. Application of OMF acts as a nutrient source for hydrocarbon degrading microbes which help in the absorption of the pollutants from the polluted soil. Therefore, PP and LL have good potentials to phytoremediate contaminants from crude oil polluted soils and are recommended.

Keywords: Phytoremediation, Native plants, Pollution, Organo-mineral fertilizer, Brassinolide

INTRODUCTION

Advances in technology coupled with natural disaster have contributed to soil degradation. Contamination of agricultural soils by crude oil is one of the most prevalent problems associated with exploration and processing of petroleum hydrocarbon (Ayotamuno *et al.,* 2006). Oil pollution is of a great concern the world over. Even at the micro-level, contamination of the environment by crude oil is a global problem in that it leads to loss of vegetation, food insecurity and biodiversity (Ijah *et al.,* 2018).

Many techniques such as soil excavation, soil washing/flushing, chemical immobilization, stabilization, electro-kinetics, covering the polluted soil

with clean soils and the dilution method have been adopted and used to clean up polluted soils. These techniques are rather costly, labour intensive and further degrade the soil (Lundstedt, 2003). Thus, biological method such as phytoremediation has been recommended as one of the most suitable methods for the remediation of hydrocarbon contaminated soils. Phytoremediation is a technique that uses plants and their associated micro-organisms to degrade, extract, contain or render harmful substances harmless in the soil (Helmisaari *et al.*, 2007). According to (Eredei *et al.*, 2005), this technology is believed to be less disruptive to the soil as well as cost-effective. Besides being easy to implement, it is eco-friendly and aesthetically pleasing than the traditional methods (Henry, 2000). These methods of remediation according to (Meagher, 2000) also prevent the excavation and transportation of pollutants from one place to another, thus, reduced the risk of spreading the contaminants. Efe and Okpali (2012) documented that, soil amendments are needed to increase microbial activities in the soil and for effective bioremediation of crude oil contaminated soil. Searching for the most effective plant species to clean up hydrocarbon polluted soils is a critical step in phytoremediation trials. Mathematical modeling has been used to evaluate the appropriate plant species (Thoma *et al.*, 2003) but in general, the selection of plant species for phytoremediation for specific sites is empirical and based on preliminary results obtained from pot experiment (Kirkpatrick *et al.*, 2006; Euliss *et al.*, 2007). According to Merkl *et al.* (2005), plant species selected for phytoremediation are required to be fast growing, hardy, possess high biomass, adaptable to local climate, compatible to soil processes and must have the ability to degrade the contaminant concerned. The use of hormone (Brassinolides) has been reported to play an important role in phytoremediation trials. Brassinolide is a plant growth hormone, derived from Brassica, a genus of plants in the mustard family (Brassicaceae). It is one of the essential plant hormones that make the root of plant stronger and improves the ability of resistance to insects and diseases. It can strengthen the ability of plants to resist harsh environmental conditions like cold, drought, contaminated soil and it improves the uptake and translocation of micro and macro nutrients, thereby increasing plant growth and development (Sasse, 1997). Considering the detrimental effects of crude oil pollution on soil and plants and its attendant implications on food security and environmental integrity, it has become necessary to source for a more cost effective, affordable, adaptable and environmentally friendly method to restore such soils back to its original state. Therefore, the objective of the study was to assess the effects of integrated use of native plant species, organo-mineral fertilizer (OF), Brassinolide for the remediation of crude oil polluted soils.

MATERIALS AND METHODS

Description of the Study Area:

The study was conducted at the Teaching and Research Farm of the Faculty of Agriculture, Akwa Ibom State University, which lies between Latitude $4^{0}30^{1}$ and $5^{0}33^{1}$N and Longitude 7^{0} 25^{1}E and $8^{0}25^{1}$E with a mean annual rainfall of over 3000 mm. Mean annual temperature ranges between 24- 30°C with relative humidity

of 75-80% within a year (Petters *et al.,* 1989).

Experimental materials used / Soil sampling and processing

The experimental materials used were crude oil, OMF, plant growth hormones (Brassinolide) and twelve native plant species. Composite soil samples (0-30 cm) depth was randomly collected from the Teaching and Research Farm of the Faculty of Agriculture, Akwa Ibom State University. The soil samples collected were air dried, crushed, sieved using a 2 mm and stored for laboratory analysis.

Treatment application and planting:

The experiment was conducted in two phases: Screen house and Field experiment. The potentials of some native plant species to remediate crude oil polluted soils were first assessed in the screen house. To each of the perforated plastic bucket (5 litres capacity) was added to 5 kg of the 2 mm sieved soils. Crude oil was added to the soil in the pot at various levels of 2.5% (147.5 ml) 5% (295 ml), 7.5% (442.5 ml) polluted (w/w) with a control at 0%. The crude oil was thoroughly mixed with the soil for even distribution and was watered to field capacity when necessary. One week after treatment application, *Jatropha* (*Jatropha curcas*) seedlings, White lead Tree (*Leuceana leucocephala)* and water leaf (*Talinum fructicosum*)) stems averaging 5 cm in height were transplanted from the nursery and one seedling was sown in each pot to a depth of 5 cm. Grasses and legumes were transplanted within the experimental area, while Gliricidia (*Gliricidia sepium) measuring* (5 cm) was planted by stem cuttings. The experiment was 13 x 4 factorial and was laid out in a completely randomized design and replicated thrice at the screen house of the Department of Soil/Crop Science, Faculty of Agriculture, Akwa Ibom State University. The pots were irrigated on the day of sowing and at regular intervals. The duration of the pot experiment was four months. Soil samples were collected per pot at 2 and 4 months after planting for laboratory analysis.

Field experiment

Two identified plant species with acceptable potentials for phytoremediation of crude oil polluted soil from pot experiment were further studied under field conditions. The experimental site was manually cleared, stumped, tilled and beds measuring 1 m x 1 m made. The experiment was laid out in a randomized complete block design with 15 treatments replicated thrice. Each block was separated from each other with an alley of 1 m and each plot by 0.5 m. Crude oil was applied to specified plots by sprinkling from perforated cans. The plots were left undisturbed for one week. After one week it was tilled and OF applied to specified plots at the rate of 5 t/ha in order to provide nitrogen which is a major limiting factor in crude oil polluted soils and also for the growth of soil microorganisms. Two weeks after application of the fertilizer, young seedlings measuring 5cm of *Leuceana leucocephala* and elephant grass (*Pennisetum purpureum*) were sown on the prepared beds to a depth of 5 cm. Twenty-eight (28) days after planting, plant growth hormones (Brassinolide) was diluted at the rate of 1 ml to 1000 ml (1litre) of distilled water and was applied at the rate of 15 ml/plant. Soil samples were collected from each plot at 3 and 6

months after pollution for laboratory analysis.

Laboratory analysis of soil samples

Determination of total petroleum hydrocarbon in soil and plants:

A modified approach published by Akpan and Usuah (2014) was used to determine the total petroleum hydrocarbon content in the polluted soil samples at 2, 3, 4 and 6 months. The hydrocarbon content in the oil -polluted soil was extracted using 10 ml of n-hexane after 10 g of soil samples were measured into a 50 ml flask. To separate the oil completely from the soil sample, the mixture was agitated violently on a magnetic stirrer for 30 minutes. A whatman filter paper was used to filter the solution and the filtrate was then diluted by adding 1 ml of the extract to 50 ml of n-hexane. Utilizing n-hexane as a blank, the absorbance of this solution was measured in a spectrophotometer at 480 nm. It was then represented as mg/kg of total hydrocarbon content.

Determination of Total petroleum hydrocarbon content in polluted test plants

The method described by Numbere (2019) was adopted for the analysis of total petroleum hydrocarbon in polluted plants. This was determined using UV-Vis spectrophotometric method. The polluted test plants were uprooted at 4 and 6 months, washed and dried in an oven at 60^0C for 24 hours to remove the moisture. After being crushed, the dried samples were weighed (2 g) in a glass beaker with 2 ml of n-hexane using a glass rod and vigorous swirling; the materials were homogenized within 30 minutes. The samples were then run through a glass funnel filled with cotton wool, silica gel and anhydrous sodium sulfate to filter out any remaining particles. After filtration, n-hexane was used as a blank and 10 ml of the filtered organic extract was placed into a 10 ml sample vial for spectrophotometer analysis at wavelength 610 nm. The amount of total hydrocarbon was given in mg/kg.

Statistical analysis:

The data obtained were subjected to analysis of variance. Duncan's Multiple Range Test (DMRT) was used to compare differences between means at 5% significant level.

RESULTS AND DISCUSSION

Properties of the crude oil and organo-mineral fertilizer used for the study

Details chemical properties of the crude oil and organo-mineral fertilizer are presented in Tables1 and 2.

Table 1: Characteristics of crude oil used for the study

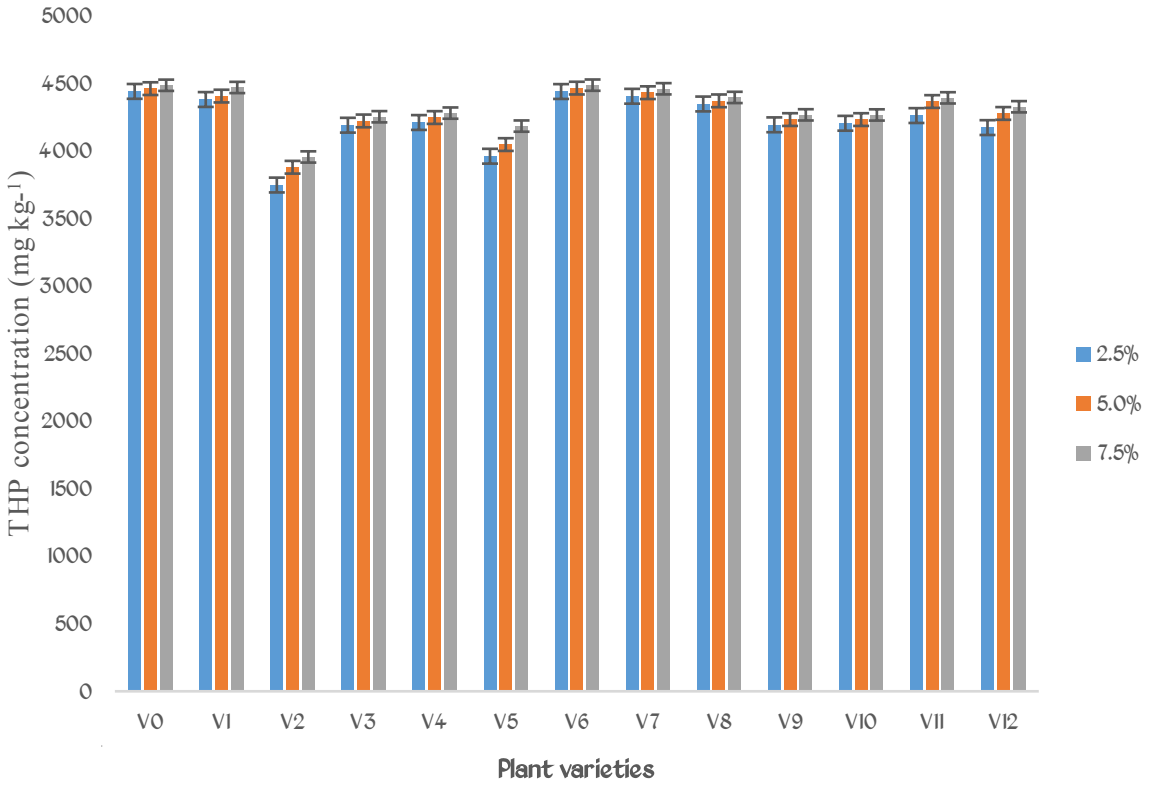
Parameter	Specific value
Specificgravity (g/cm^3)	0.834
Viscosity (CP)	0.28
Carbon (%)	85.5
Hydrogen (%)	12.61
Sulphur (%)	1.48
Nitrogen (%)	0.47
Oxygen (%)	0.50
Trace metals (%)	0.13
Gas : oil ratio	88.1

Table 2: Chemical analysis of organo-mineral fertilizer used for the field experiment

Properties	Values
N (%)	2.8
P (%)	1.2
K (%)	2.2
Moisture (%)	14
Total organic matter (%)	40

Total petroleum hydrocarbon content in soil in the screen house

The results of total petroleum hydrocarbon (TPH) content in soils treated with different concentration of crude oil and planted with different plant species are shown in Figure 1. At 2 months after treatment (MAT), there was no significant change in TPH among soils treated with different plant species (Figure 1). However, the level of TPH was lowest in soils under *PP* and *LL treatments*. Although both species cause a reduction in soil TPH, they have demonstrable potentials as shown by the lesser amount of residual crude in the soil compared with other soils that received the same amount of crude oil.



V_O = unpolluted & unplanted soil, V_1= Carpet grass(*Axonopus compressus*), V_2= Elephant grass(*Pennisetum purpureum*), V_3=Goose weed (*Eleusine indica*), V_4= Guinea grass(*Panicum maximum*), V_5=White leadtree (*Leuceana leucocephala*), V_6= Gliricidia(*Gliricidia sepium*), V_7= Waterleaf(*Talinum fructicosum*), V_8= Siam weed(*Chromoleana odorata*), V_9= Nut Sedge weed(*Cyperus rotundus*), V_{10}= Calapo(*Calapogonium mucunoides*), V_{11}= Jatropha(*Jatropha curcas*), V_{12}= Centro(*Centrosema pubescens*).

Figure 1: Total petroleum hydrocarbon (TPH) in soils at 2 months after crude oil pollution in the greenhouse

The result of total petroleum hydrocarbon (TPH) content in soils treated with different concentrations of crude oil at 4 months after pollution in the screen house are shown in Figure 2. At 4 MAT, there was a reduction in TPH in soils under *PP* and *LL.* However, there were no significant differences recorded among the plants at 4 months. The reduction was attributed to fast growth rate of these plants species, high biomass and high population of hydrocarbon degraders in the soil.

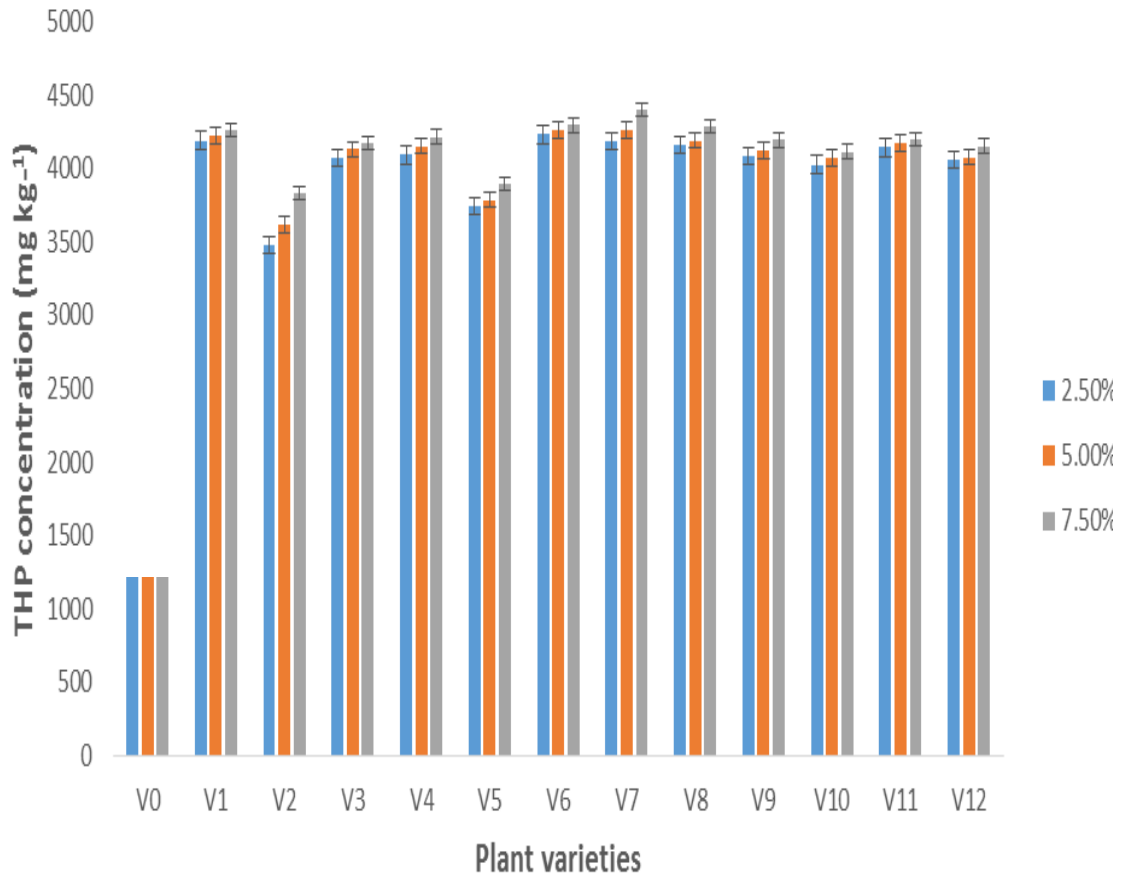


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Figure 2: Total petroleum hydrocarbon (TPH) content in soils at 4 months after pollution in the greenhouses

Total petroleum hydrocarbon in plant tissues at 4 months:

The amount of total petroleum hydrocarbon absorbed by *PP and LL* in soil polluted with 2.5 % crude oil was significantly ($P < 0.05$) higher than the other plant species after 4 months (Figure 3). This was followed by soil with 5.0 % and 7.5% pollution. The lowest TPH was in *G. sepium, T. fructicosum, C. odorata, A. compressus, C. cyperus, C. mucunoides, J. curcas and C. pubescens.* The highest accumulation of TPH in *PP* and *LL* may be attributed to their agronomic advantages such as adaptability to various soil types, rapid growth rate and higher root biomass which increased the secretion of exudates such as phenol to stimulate microbial activity in the soil. Besides, these two species were shown to maintain a large number of soil microorganisms such as petroleum hydrocarbon degraders. This result is in consonance with the findings of Edwin-Wosu (2010) and Noori *et al.* (2012) who reported higher reduction of total petroleum hydrocarbon in soils planted with *LL* and some species of the Fabaceae family. Given the result obtained from the pot experiment, *PP* and *LL* were selected based on their potential to remediate crude oil polluted soil in the field.

Total petroleum hydrocarbon content in the field

The total petroleum hydrocarbon content of soils amended with organo-mineral fertilizer and Brassinolide under *P. purpureum* and *L. leucocephala* was significantly ($P < 0.05$) lower than other treatments at 3 and 6 months after pollution (Figure 4). The decrease in TPH in soils planted and amended with organo-mineral fertilizer and Brassinolide could be attributed to ample supply of necessary nutrients for microbial growth and degradation of TPH.

At 6 months after pollution, the highest reduction in TPH across polluted soil was in soil amended with organo-mineral fertilizer and Brassinolide under *P. purpureum* ond *L. leucocephala*. The possible reason for the low content of TPH in the polluted but amended and planted soils might be due to such mechanisms as (Rhizo degradation) which involve the interaction effect of plant and soil microorganisms that favoured a greater reduction in total hydrocarbon. Also, the ability of the treatments (organo-mineral fertilizer and Brassinolide) to supply the soil microbes and the different plant species with nutrients such as nitrogen and carbon for their growth and development might also be a possible reason for the low content. This result agrees with that of White *et al.* (2008) who reported lower total petroleum hydrocarbon in vegetated fertilized plots than non-vegetated non-fertilizer plots.

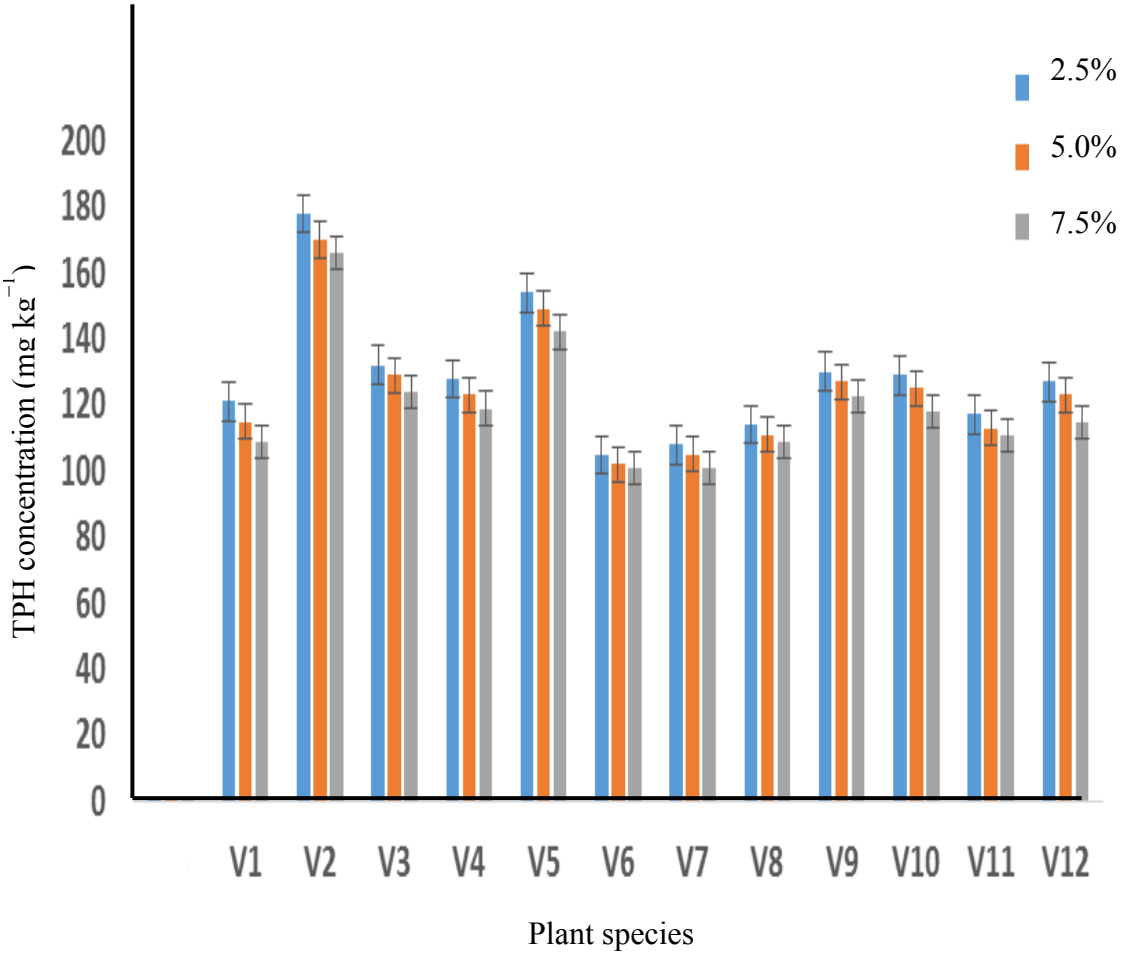

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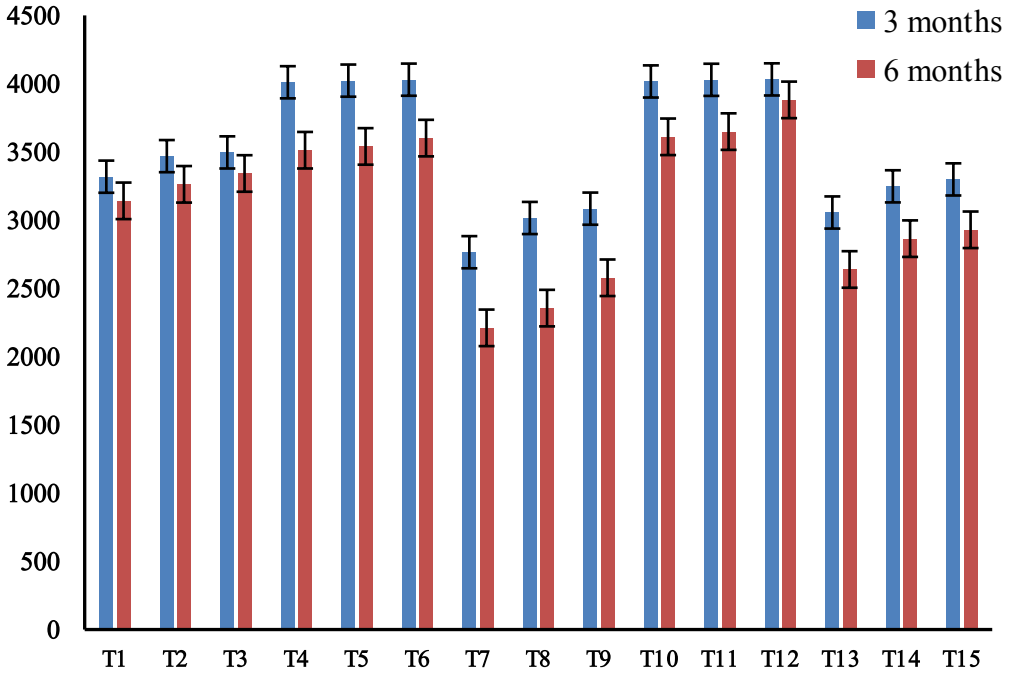
Figure 3: Total petroleum hydrocarbon content in plant tissues at 4 months after pollution in the greenhouse



T_1 = 2.5 % crude oil polluted soil + organo-mineral fertilizer (OF), no planting or Brassinolide
T_2 = 5.0 % crude oil polluted soil + OF, no planting of Brassinolide
T_3 = 7.5 % crude oil polluted soil + OF, no planting or Brassinolide
T_4 = 2.5 % crude oil polluted soil + no OF or Brassinolide under *Pennisetum purpureum*
T_5 = 5.0 % crude oil polluted soil + no OF or Brassinolide under *P.purpureum*
T_6 = 7.5 % crude oil polluted soil + no OF or Brassinolide under *P. purpureum*
T_7 = 2.5 % crude oil polluted soil + OF+ Brassinolide under *P.purpureum*
T_8 = 5.0 % crude oil polluted soil + OF + Brassinolide under *P.purpureum*
T_9 = 7.5 % crude oil polluted soil + OF + Brassinolide under *P. purpureum*
T_{10} = 2.5 % crude oil polluted soil + no OF or Brassinolide under *Leuceana leucocephala*
T_{11} = 5.0 % crude oil polluted soil + no OF or Brassinolide under *L. leucocephala*
T_{12} = 7.5 % crude oil polluted soil + no OF or Brassinolide under *L. leucocephala*
T_{13} = 2.5 % crude oil polluted soil + OF + Brassinolide under *L. leucocephala*
T_{14} = 5.0 % crude oil polluted soil + OF + Brassinolide under *L.leucocephala*
T_{15} = 7.5 % crude oil polluted soil+ OF + Brassinolide under *L. leucocephala*

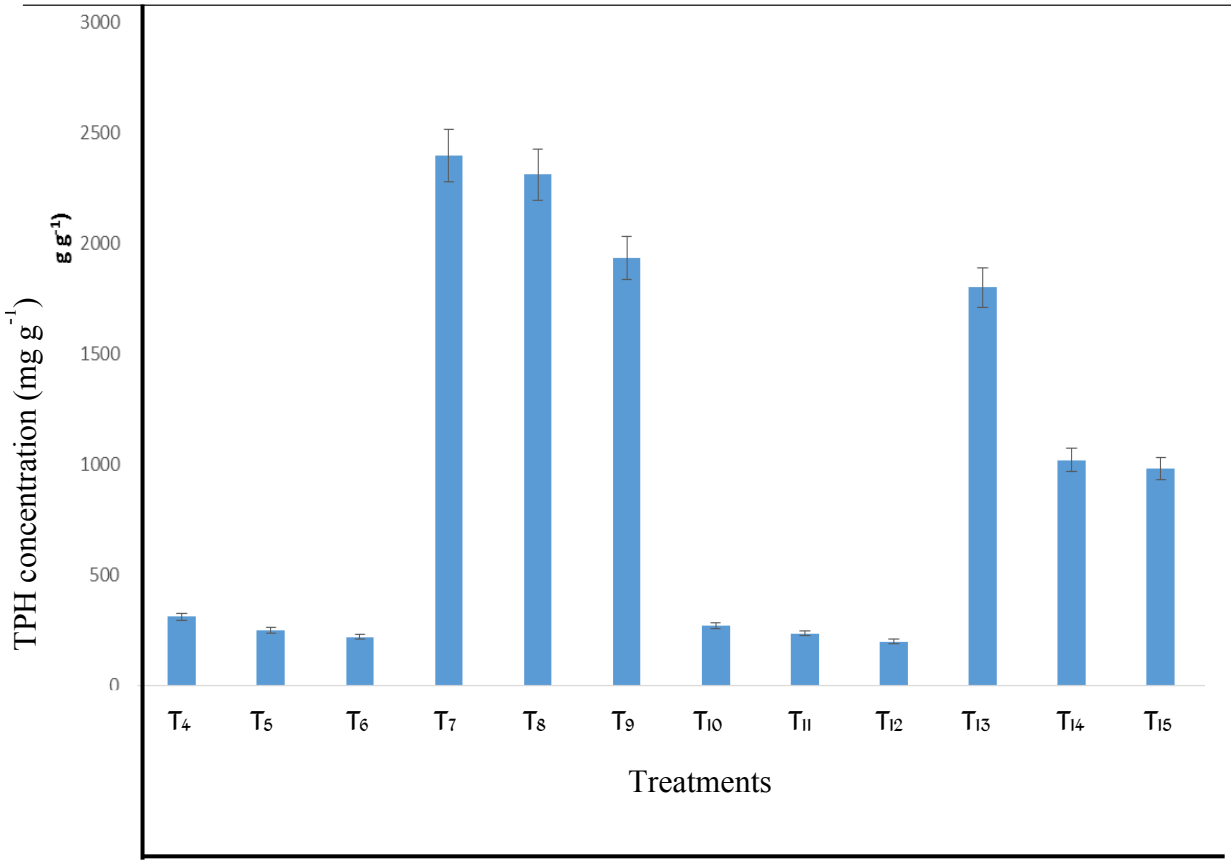
Figure 4: Total Petroleum hydrocarbon (TPH) content in soil at 3 and 6 months after crude oil pollution in the field

Total petroleum hydrocarbon content in plants at 6 months after crude oil pollution in the field

Pennisetum purpureum planted in soil polluted with 2.5 %, 5.0 % crude oil amended with organo-mineral fertilizer and brassinolide (T_7 and T_8) accumulated higher levels of TPH than other plants (Figure 5). This was followed by *P. purpureum* planted in soils polluted with 7.5 % crude oil, amended with organo-mineral fertilizer and brassinolide (T_9) although there were not significantly ($P < 0.05$) different from *L. leuceana* polluted with 2.5 % crude oil, amended with organo-mineral fetilizer and brassinolide (T_{13}).The result obtained from this study confirmed previous findings by Xia (2004), Muratova *et al.* (2008), Bordoloi *et al.* (2012) and Budhadev *et al.* (2012) who reported higher reduction of petroleum hydrocarbon in vegetated amended soils than in non-vegetated and unammended soil. The higher removal of crude oil observed in this study conforms with the report by Edwin-Wosu(2000) and Xia (2004) who listed *Pennisetum purpureum* and *Leuceana leucocephala* as plants that can accumulate higher levels of hydrocarbon from crude oil polluted soil than other plants. The removal of crude oil by these plants may be attributed to several mechanisms of phytoremediation such as: rhizodegradation (interaction of the plant with bacteria and fungi) (McCutcheon *et al.*, 2003; Siciliano *et al.*, 2003), phytoextraction (process in which metal accumulating plants are used to transport and concentrate metals from the soil into the harvestable parts of roots and above ground shoots) (Morikawa *et al.*, 2003; Sinha *et al.*, 2004) and Phytovolatilization (McCutcheon *et al.*, 2003). An evidence of the process of phytovolatilization was the leaf burn (leaf chlorosis) observed in the plants during the first few weeks of remediation. This suggests that violatile organic compounds were taken up by the roots of the plants, translocated within the plants and transpired via the stems and leaves (Wiltse *et al.*, 1998). The leave burn gradually disappear in the course of remediation meaning that so many volatile petroleum hydrocarbons had been transferred to the atmosphere.

Conclusion and recommendation

In conclusion, *Pennisetum purpureum* and *Leuceana leucocephala* showed the ability to clean up crude oil polluted soils. This is confirmed by the higher concentration of total petroleum hydrocarbon in plant and greater reduction in the soil at 3 months (2750 $mgkg^{-1}$) and at 6 months (2250 $mgkg^{-1}$). *Pennisetum purpureum* had higher level of total petroleum hydrocarbon in their tissues (2500 $mgkg^{-1}$) and low in soil indicating its remediating potentials over other species of plants. Since these two plant species are widely distributed and have proved successful in phytoremediation of crude oil polluted soils, thus, it is recommended for many tropical countries facing the problem of crude oil pollution.



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T_2 = 5.0 % crude oil polluted soil + OF, no planting of Brassinolide
T_3 = 7.5 % crude oil polluted soil + OF, no planting or Brassinolide
T_4 = 2.5 % crude oil polluted soil + no OF or Brassinolide under *Pennisetum purpureum*
T_5 = 5.0 % crude oil polluted soil + no OF or Brassinolide under *P.purpureum*
T_6 = 7.5 % crude oil polluted soil + no OF or Brassinolide under *P. purpureum*
T_7 = 2.5 % crude oil polluted soil + OF+ Brassinolide under *P.purpureum*
T_8 = 5.0 % crude oil polluted soil + OF + Brassinolide under *P.purpureum*
T_9 = 7.5 % crude oil polluted soil + OF + Brassinolide under *P. purpureum*
T_{10} = 2.5 % crude oil polluted soil + no OF or Brassinolide under *Leuceana leucocephala*
T_{11} = 5.0 % crude oil polluted soil + no OF or Brassinolide under *L. leucocephala*
T_{12} = 7.5 % crude oil polluted soil + no OF or Brassinolide under *L. leucocephala*
T_{13} = 2.5 % crude oil polluted soil + OF + Brassinolide under *L. leucocephala*
T_{14} = 5.0 % crude oil polluted soil + OF + Brassinolide under *L.leucocephala*
T_{15} = 7.5 % crude oil polluted soil+ OF + Brassinolide under *L. leucocephala*

Figure 5: Total petroleum hydrocarbon content in plants at 6 months after crude oil pollution in the field

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